WEST Search History

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updated 8/9/04

DATE: Monday, August 09, 2004

Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	(0-157$ or 0157 or o157 or o-157).clm.	49
☐	L2	L1 same shiga$.clm.	2
☐	L3	L1 and ($dihydrazide or $di-hydrazide).clm.	0
☐	L4	L1 and (carrier or linker or conjugate or conjugated or bound or binder or linked or linking or conjugating or coupling or coupled or couple or joiner or joined or attach or attachment or attaching or attached).clm.	15
☐	L5	szu.in.	279
☐	L6	L5 and (coli.clm. or ehec.clm. or etec.clm.)	0
☐	L7	L5 and coli.clm.	0
☐	L8	kondau.in. or robbins!.in.	2153
☐	L9	L8 and l1	0
☐	L10	L8 and $saccharide.clm.	12
☐	L11	L8 and fruit	22
☐	L12	l5 or l8	2431
☐	L13	L12 and coli	26
☐	L14	('5738855' |'5445817' |'6685949' |'6632437')!.PN.	4
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L15	L12 and coli	26
☐	L16	L12 and coli	26
☐	L17	kondau.in. or robbins!.in.	4787
☐	L18	szu.in.	602
☐	L19	L18 or l17	5381
☐	L20	L19 and (o157 or 0157 or o-157 or o1-57 or 01-57 or 0157h7 or o157h7 or h7).ti,ab,clm.	1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 12 of 12 returned.

1. 6685949. 05 Jul 00; 03 Feb 04. Lipooligosaccharide based vaccine for prevention of moraxella (branhamella)catarrhalis infections in humans. Gu; Xin-Xing, et al. 424/251.1; 424/184.1 424/197.11 424/234.1 424/9.2 435/170 435/197 435/72 435/97. A61K039/02 A61K039/00 A01N065/00 C12P019/00 C12P019/18.

2. 6632437. 15 Nov 93; 14 Oct 03. Immunogenic polysaccharide-protein conjugates containing poly .alpha.(2.fwdarw.8), .alpha.(2.fwdarw.9) NeuNAc capsular polysaccharides. Schneerson; Rachel, et al. 424/193.1; 424/137.1 424/194.1 424/197.11 424/241.1 530/403 530/404 530/405 530/406. A61K039/385 A61K039/395 A61K039/108 C08H001/00.

3. 6607725. 20 Feb 01; 19 Aug 03. Conjugate vaccine for nontypeable Haemophilus influenzae. Gu; Xin-Xing, et al. 424/184.1; 424/178.1 424/183.1 424/193.1 424/201.1 424/202.1 424/203.1 424/234.1 424/256.1 424/270.1 435/183 435/271 435/274 435/69.3 435/851. A61K039/395 A61K039/40 A61K039/42 A61K039/44 A61K039/00.

4. 6358724. 18 Jun 01; 19 Mar 02. Isolation and composition of novel glycosidases. Wong-Madden; Sharon T., et al. 435/207; 435/18 435/200 435/243 435/252.1 435/4. C12N009/38.

5. 6342365. 24 Feb 99; 29 Jan 02. Isolation and composition of novel glycosidases. Wong-Madden; Sharon T., et al. 435/18; 435/183 435/200 435/201 435/207 435/208 435/4 435/7.4 435/7.6 435/7.72. C12Q001/34 C12Q001/00 G01N033/573 C12N009/00 C12N009/28.

6. 6207157. 23 Apr 97; 27 Mar 01. Conjugate vaccine for nontypeable Haemophilus influenzae. Gu; Xin-Xing, et al. 424/184.1; 424/197.11 424/250.1 424/251.1 424/256.1 424/262.1 424/94.63 435/197 435/252.3 435/97. A61K039/00 A61K038/43 A61K038/48 A61K039/385.

7. 6203999. 14 Jan 99; 20 Mar 01. Detection of prostate and other cancers by assaying for cancer-specific antigens having linked oligosaccharides which are at least triantennary. Robbins; Phillips W., et al. 435/7.21; 435/7.23. G01N033/567.

8. 5902725. 02 Jul 97; 11 May 99. Detection of prostate and other cancers by assaying for cancer-specific antigens having linked oligosaccharides which are at least triantennary. Robbins; Phillips W., et al. 435/7.1; 435/7.21 435/7.23 436/63 436/64. G01N033/53.

9. 5770405. 24 Jun 96; 23 Jun 98. Isolation and composition of novel glycosidases. Wong-Madden; Sharon T., et al. 435/74; 435/200 435/208 435/910 536/23.2. C12P019/44 C12N009/24 C12N009/40 C12N001/00.

10. 5445817. 21 Aug 92; 29 Aug 95. Pertussis toxin used as a carrier protein with non-charged saccharides in conjugate vaccines. Schneerson; Rachel, et al. 424/194.1; 424/197.11 424/240.1 424/244.1 424/831 530/402 530/403. A61K039/385 A61K039/09 A61K039/10.

11. 5204098. 16 Feb 88; 20 Apr 93. Polysaccharide-protein conjugates. Szu; Shousun C., et al. 424/194.1; 424/197.11 424/258.1 424/831 435/961 530/391.1 530/395 530/404 530/408 530/807. A61K039/112 A61K039/385 C07K017/10.

☐ 12. 4386026. 20 Apr 81; 31 May 83. Cell-specific glycopeptide ligands. Ponpipom; Mitree M., et al. 536/53; 435/184 530/322 536/115 536/18.7 536/54. C07C103/52 C08B037/00 C07H011/00.

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Terms	Documents
L8 and $saccharide.clm.	12

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Search Results - Record(s) 1 through 4 of 4 returned.

L14: Entry 1 of 4 File: USPT Feb 3, 2004

US-PAT-NO: 6685949
DOCUMENT-IDENTIFIER: US 6685949 B1

TITLE: Lipooligosaccharide based vaccine for prevention of moraxella (branhamella) catarrhalis infections in humans

DATE-ISSUED: February 3, 2004

US-CL-CURRENT: 424/251.1; 424/184.1, 424/197.11, 424/234.1, 424/9.2, 435/170, 435/197, 435/72, 435/97

INT-CL: [07] A61 K 39/02, A61 K 39/00, A01 N 65/00, C12 P 19/00, C12 P 19/18

L14: Entry 2 of 4 File: USPT Oct 14, 2003

US-PAT-NO: 6632437
DOCUMENT-IDENTIFIER: US 6632437 B1

TITLE: Immunogenic polysaccharide-protein conjugates containing poly .alpha. (2.fwdarw.8), .alpha.(2.fwdarw.9) NeuNAc capsular polysaccharides

DATE-ISSUED: October 14, 2003

US-CL-CURRENT: 424/193.1; 424/137.1, 424/194.1, 424/197.11, 424/241.1, 530/403, 530/404, 530/405, 530/406

INT-CL: [07] A61 K 39/385, A61 K 39/395, A61 K 39/108, C08 H 1/00

L14: Entry 3 of 4 File: USPT Apr 14, 1998

US-PAT-NO: 5738855
DOCUMENT-IDENTIFIER: US 5738855 A

TITLE: Synthesis of typhoid fever vaccine from a plant or fruit polysaccharide

DATE-ISSUED: April 14, 1998

US-CL-CURRENT: 424/258.1; 424/184.1, 424/192.1, 424/195.11, 424/236.1, 424/725, 424/777, 514/2, 514/53, 530/402, 536/123

INT-CL: [06] A61 K 39/00, A61 K 39/385, A61 K 45/00, C07 G 17/00

L14: Entry 4 of 4 File: USPT Aug 29, 1995

US-PAT-NO: 5445817
DOCUMENT-IDENTIFIER: US 5445817 A

TITLE: Pertussis toxin used as a carrier protein with non-charged saccharides in

conjugate vaccines

DATE-ISSUED: August 29, 1995

US-CL-CURRENT: 424/194.1; 424/197.11, 424/240.1, 424/244.1, 424/831, 530/402, 530/403

INT-CL: [06] A61 K 39/385, A61 K 39/09, A61 K 39/10

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L20: Entry 1 of 1 File: DWPI Oct 30, 2003

DERWENT-ACC-NO: 2000-195083
DERWENT-WEEK: 200382

TITLE: New conjugate of bacterial O-specific polysaccharide, used in vaccines against infection by hemolytic-uremic Escherichia coli, contains covalently linked Shiga toxin component

INVENTOR: KONADU, E; KONADU, Y A ; ROBBINS, J B ; SZU, S C

PATENT-ASSIGNEE: US DEPT HEALTH & HUMAN SERVICES (USSH)

PRIORITY-DATA: 1998WO-US14976 (July 20, 1998)

Search Selected Search ALL Clear

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 767047 B	October 30, 2003		000	A61K039/385
WO 200004922 A1	February 3, 2000	E	043	A61K039/385
AU 9885758 A	February 14, 2000		000	A61K039/385
BR 9815953 A	March 6, 2001		000	A61K039/385

DESIGNATED-STATES: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
AU 767047B	July 20, 1998	1998AU-0085758	
AU 767047B		AU 9885758	Previous Publ.
AU 767047B		WO 200004922	Based on
WO 200004922A1	July 20, 1998	1998WO-US14976	
AU 9885758A	July 20, 1998	1998AU-0085758	
AU 9885758A	July 20, 1998	1998WO-US14976	
AU 9885758A		WO 200004922	Based on
BR 9815953A	July 20, 1998	1998BR-0015953	
BR 9815953A	July 20, 1998	1998WO-US14976	
BR 9815953A		WO 200004922	Based on

INT-CL (IPC): A61 K 39/385

ABSTRACTED-PUB-NO: WO 200004922A
BASIC-ABSTRACT:

NOVELTY - Conjugate (I) comprises an O-specific polysaccharide (II) covalently bound to a carrier (III), which is the B-subunit of Shiga toxin 1 or 2, or a non-toxic mutant Shiga 1 or 2 holotoxin. (II) is from the Eschericia coli strain O157, forming conjugate (Ia), or from E. coli strains O111, O17 or O26, or from Shigella dysenteriae.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a pharmaceutical composition comprising (I) and a carrier;

(2) a vaccine comprising a conjugate of (II), from O157, and a carrier protein (III), in a carrier;

(3) a method of inducing serum antibodies which are bacteriostatic or bacteriocidal to E. coli O157, in a mammal, comprising administering (I) in a carrier;

(4) a method of passively immunizing a mammal against E. coli O157 infection, comprising administering the composition of (1) or (2);

(5) a composition comprising antibodies (Ab1) immunoreactive with (II) from O157;

(6) Ab1; and

(7) a composition comprising antibodies (Ab2) immunoreactive with Shiga toxin 1 or 2.

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - (I) induce serum antibodies that are bacteriostatic or bactericidal against E. coli O157. Mice were immunized subcutaneously with 3 doses (14 day intervals) of (II) (from O157)-Shiga toxin 1 B subunit conjugate (2.5 mu g (II)). Their sera then provided over 99% neutralization of Shiga toxin 1 at dilution 1:100, 98% at 1:1000 and 70% at 1:10000, but did not neutralize Shiga 2 toxin. The same treatment induced significant levels of antibodies against lipopolysaccharide, e.g. titers (in enzyme-linked immunosorbant assay) of 0.63 for IgG and 0.14 for IgM.

USE - (Ia) are used as vaccines to protect against infection by E. coli O157 or other strains that cause hemolytic-uremic syndrome. Antibodies raised against (Ia) are useful for passive immunization, for treatment or protection.

ADVANTAGE - (I) induces both bactericidal antibodies against O157 and antibodies against Shiga toxin. These antibodies inactivate O157 at the entrance to the jejunum, before infection is established.

ABSTRACTED-PUB-NO: WO 200004922A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/0

DERWENT-CLASS: B04 D16
CPI-CODES: B04-C02; B04-F10; B04-N03; D05-C08; D05-H07; D05-H09; D05-H11; D05-H13;

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L10: Entry 1 of 12 File: USPT Feb 3, 2004

US-PAT-NO: 6685949
DOCUMENT-IDENTIFIER: US 6685949 B1

TITLE: Lipooligosaccharide based vaccine for prevention of moraxella (branhamella) catarrhalis infections in humans

DATE-ISSUED: February 3, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gu; Xin-Xing	Rockville	MD		
Robbins; John B.	Chevy Chase	MD		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The United States of America as represented by the Department of Health & Human Services	Washington	DC			06

APPL-NO: 09/ 610034 [PALM]
DATE FILED: July 5, 2000

PARENT-CASE:
RELATED APPLICATIONS This application is a continuation and claims the benefit of priority of International Application No. PCT/US99/00590 filed Jan. 12, 1999, designating the United States of America and published in English, which claims the benefit of priority of U.S. Provisional Application No. 60/071,483 filed Jan. 13, 1998, both of which are hereby expressly incorporated by reference in their entireties.

INT-CL: [07] A61 K 39/02, A61 K 39/00, A01 N 65/00, C12 P 19/00, C12 P 19/18

US-CL-ISSUED: 424/251.1; 424/197.11, 424/9.2, 424/184.1, 424/234.1, 435/72, 435/97, 435/170, 435/197
US-CL-CURRENT: 424/251.1; 424/184.1, 424/197.11, 424/234.1, 424/9.2, 435/170, 435/197, 435/72, 435/97

FIELD-OF-SEARCH: 424/251.1, 424/234.1, 424/197.11, 424/9.2, 424/184.1, 435/72, 435/170, 435/97, 435/197

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5013661	May 1991	Munford et al.	435/197
☐	5334379	August 1994	Pillai et al.	424/85.2
☐	5556755	September 1996	Murphy	435/6
☐	5607846	March 1997	Murphy et al.	435/69.3
☐	5712118	January 1998	Murphy	435/69.1
☐	5725862	March 1998	Murphy	424/251.1
☐	6207157	March 2001	Gu et al.	424/184.1

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
98/53851	December 1998	WO	

OTHER PUBLICATIONS

Gu et al. , "Detoxified Lipooligosaccaride from Nontypeable Haemophilus influeanzae Conjugated to Proteins Confers Prtection agaist Otitis Media in Chinchillas." Infection and immunity, vol. 65, No. 11 , pp. 4488-4493, Nov. 1997.*

Chen, D. et al. "Evaluation of Purified UspA from Moraxella catarrhalis as a Vaccine Vaccine in a Murine Model after Active Immunization", Infection and Immunity, vol. 64, No. 6 , pp. 1900-1905, Jun. 1996.*

Gu et al. Quantitation and Biological Properties of Released and Cell- Bound Lipooligosaccharide from Nontypeable Haemophilus influenzae, . , Infection and immunity, vol. 63, No. 10 , pp. 4115-4120, Oct. 1995.*

Gu et al. , Preparation, Characterization and Immunogenicity of Meningococcal Lipooloigosaccharide- Derived Oligosaccaharide- Protein Conjugates, Infection and immunity, vol. 61, No. 5 , pp. 1873-1880, May 1993.*

Gu et al. Synthesis , Characterization, and Imunologic Properties of Detoxified Lipooligosaccharide from Nontypeable Haemophilus influenzae Conjugated to Proteins, Infection and immunity, vol. 64, No. 10 , pp. 4047-4053, Oct. 1996.*

Vaneechoutte et al. , "Serological Typing of Branhamela catarrhalis Strains on the Basis of Liopolysaccharide Antigen", Journal of Clinical Microbiology, vol. 28, No. 2, pp. 182-187, 1990.*

Campagnari et al., "Lipooligosaccharide epitopes shared smong Gram- negative non-enteric mucosal pathogens" Microbial Pathogenesis, vol. 8, pp. 353-362, 1990.*

Erwin, et al. (1991) Enzymatically Deacylated Neisseria Lipopolysaccharide (LPS) Inhibits Murine Splenocyte Mitogenesis induced by LPS, Infection and Immunity 59(6): (6): 1881-1887.

Green, et al. (1994) Nontypeable Haemophilus influenzae Lipo-oligosaccharide Conjugates as Vaccine Candidates against NIHi, Vaccines94, Modern Approaches to New Vaccines Including Prevention of AIDS: 125-129.

Gupta, et al. (1992) Synthesis, Characterization, and Some Immunological Properties of Conjugates Composed of the detoxified Lipopolysaccharide of Vibrio cholerae O1 Serotype Inaba Bound to Cholera Toxin. Infection and Immunity 60(8):3201-3208.

Gupta, et al. (1995) Comparative Immunogenicity of Conjugates Composed of Escherichia coli O111 O-Specific Polysaccharide, Prepared by Treatment with Acetic Acid or Hydrazine, Bound to Tetanus Toxoid by Two Synthetic Schemes. Infection and Immunity 63(8):2805-2810.

Konadu, et al. (1994) Preparation, Characterization, and Immunological Properties in in Mice of Escherichia coli O157 O-Specific Polysaccharide-Protein Conjugate Vaccines. Infection and Immunology 61(11):5048-5054.

Konadu, et al. (1996) Synthesis, Characterization, and Immunological Properties in

Mice of Conjugates Composed of Detoxified Lipopolysaccharide of Salmonella paratyphi paratyphi A Bound to Tetanus Toxiod, with Emphasis on the Role of O Acetyls. Infection and Immunity 64(7):2709-2715.
Polotsky, et al. (1994) Comparison of Conjugates Composed of Lipopolysaccharide from from Shigella flexneri Type 2a Detoxified by Two Methods and Bound to Tetanus Toxoid. Infection and Immunity 62(1):210-214.
Ahmed, K., et al. (1991) Possible presence of a capsule in Branhamella catarrhalis. Microbiol. Immunol. 35: 361-366.
Barenkamp, S.J. (1996) Immunization with high-molecular-weight adhesion proteins of nontypeable Haemophilus influenzae modifies experimental otitis media in chinchillas, Infect. Immun. 64: 1246-1251.
Bhushan, R., et al. (1994) Molecular cloning and characterization of outer membrane protein E of Moraxella (Branhamella) catarrhalis. J. Bacteriol. 176:6636-6643.
Blueston, C.D. (1986) Otitis media and sinusitis in children. Role of Branhamella catarrhalis. Drugs 31 (Suppl. 3): 132-141.
Boyle, F.M., et al., (1991) Branhamella (Moraxella) catarrhalis: pathogenic significance in respiratory infection. Med. J. Aust. 154:592-596.
Campagnari, A.A., et al., (1994) Growth of Moraxella catarrhalis with human transferrin and lactoferrin: expression of iron-repressible proteins without siderophore production. Infect. Immun. 62:4909-4914.
Catlin, B.W. (1990) Branhamella catarrhalis: an organism gaining respect as a pathogen. Clin. Microbiol. Rev. 3:293-320.
Chapman, A.J., Jr., et al. (1985) Development of bactericidal antibody during Branhamella catarrhalis infection. J. Infect. Dis. 151:878-882.
Chen, D., et al., (1996) Evaluation of purified UspA from Moraxella catarrhalis as a a vaccine in a murine model after active immunization. Infect. Immun. 64:1900-1905.

Christensen, J.J., et al., (1996) Serum antibody response to outer membrane proteins proteins of Moraxella (Branhamella) catarrhalis in patients with bronchopulmonary infection. Clin. diagn. Lab. Immunol. 3:717-721.
Cohen, D., et al. (1997) Double-blind vaccine-controlled randomised efficacy trial of an investigational Shigella sonnei conjugate vaccine in young adults. Lancet 340:155-159.
Doern, G.V. (1986) Branhamella catarrhalis--an emerging human pathogen. Diagn. Microbiol. Infect. Dis. 4: 191-201.
Doyle, W.J. (1989) Animal models of otitis media: other pathogens. Pediatr. Infect. Dis J. 8:Suppl. 45-47.
Edebrink, P., et al. (1994) Structural studies of the O-polysaccharide from the lipopolysaccharide of Moraxella (Branhamella) catarrhalis serotype A (strain ATCC 25238). Carbohydr. Res. 257:269-284.
Edebrink, P., et al. (1995) Structural studies of the O-antigen oligosaccharides from two strains of Moraxella catarrhalis serotype C. Carbohydr. Res. 266:237-261.
Edebrink, P., et al. (1996) The structures of oligosaccharides isolated from the lipopolysaccharide of Moraxella catarrhalis serotype B, strain CCUG 3292. Carbohydr. Carbohydr. Res. 295: 127-146.
Ejlertsen, T., et al. (1994) Branhamella catarrhalis in children and adults. A study study of prevalence, time of colonisation, and association with upper and lower respiratory tract infections. J. Infect. 29:23-31.
Eliasson, I. (1986) Serological identification of Branhamella catarrhalis. Serological evidence for infection. Drugs 31(Suppl. 3):7-10.
Enright, M.C., et al. (1997) Moraxella (Branhamella) catarrhalis-clinical and molecular aspects of a rediscovered pathogen. J. Med. Micro-biol. 46:360-371.
Faden, H., et al. (1994) Epidemiology of Moraxella catarrhalis in children during the first 2 years of life: relationship to otitis media. J. Infect. Dis. 169:1312-1317.
Fomsgaard, J. S., et al. (1991) Comparative immunochemistry of lipopolysaccharides from Branhamella catarrhalis strains. Infect. Immun. 59:3346-3349.
Fung, C.P., et al. (1992) The antimicrobial susceptibility of Moraxella catarrhalis isolated in England and Scotland in 1991. J. Antimicrob. Chemother. 30:47-55.
Goldblatt, D., et al. (1990) Branhamella catarrhalis: antigenic determinants and the

the development of the IgG subclass response in childhood. J. Infect. Dis. 162:1128-162:1128-1135.

Green, B.A., et al. (1994) Nontype Haemophilus influenzae Lipo-oligosaccharide Conjugates as Vaccine Candidates against NTHi, p. 125-129. In E. Norrby. F. Brown, R.M. Chanock, and H.S. Ginsberg (ed), Vaccines 94. Cold Spring Harbor Laboratory Press, Plainview, N.Y.

Gu, X.-X., et al. (1997) Detoxified lipooligosaccharide from nontypeable Haemophilus Haemophilus influenzae conjugated to proteins confers protection against otitis media in chinchillas. Infect. Immun. 65:4488-4493.

Gu, X.-X., et al. (1993) Preparation, characterization, and immunogenicity of meningococcal lipooligosaccharide-derived oligosaccharide-protein conjugates. Infect. Immun. 61:1873-1880.

Gu, X.-X., et al. (1998) Synthesis and Characterization of Lipooligosaccharide-Based Based Conjugates as Vaccine Candidates for Moraxella (Branhamella) catarrhalis. Infect. Immun. 66:1891-1897.

Gu, X.-X., et al. (1995) Quantitation and biological properties of released and cell-bound lipooligosaccharides from nontypeable Haemophilus influenzae. Infect. Immun. 63:4115-4120.

Gu, X.-X., et al. (1996) Synthesis, characterization, and immunological properties of detoxified lipooligosaccharide from nontypeable Haemophilus influenzae conjugated conjugated to proteins. Infect. Immun. 64:4047-4053.

Gupta, R.K., et al. (1992) Synthesis, characterization, and immunological properties properties of conjugates composed of the detoxified lipopolysaccharide of Vibrio cholarae O1 serotype Inaba bound to cholera toxin. Infect. Immun. 60:3201-3208.

Helminen, M.E., et al. (1993) A major outer membrane protein of Moraxella catarrhalis is a target for antibodies that enhance pulmonary clearance of the pathogen in an animal mode. Infect. Immun. 61:2003-2010.

Helminen, M.E., et al. (1994) A large, antigenically conserved protein on the surface of Moraxella catarrhalis is a target for protective antibodies. J. Infect. Dis. 170:867-872.

Hochstein, H.D., et al. (1973) Further developments of Limulus amebocyte lysate test. Bull. Paraenter. Drug Assoc. 27:39-148.

Hu, W.-G., et al. (2000) Enhancement of Clearance of Bacteria from Murine Lungs by Immunization with Detoxified Lipooligosaccharide from Moraxella catarrhalis Conjugated to Proteins. Infect. Immun. 68:4980-4985.

Jennings, H.J., et al. (1984) Conjugation of meningococcal Lipopolysaccharide R-type type oligosaccharides to tetanus toxoid as route to a potential vaccine against group B Neisseria meningitidis. Infect. Immun. 43:407-412.

Kelly, J., et al. (1996) Separation and Characterization of O-Deacylated Lipooligosaccharides and Glycans Derived from Moraxella catarrhalis Using Capillary Electrophoresis-electrospray Mass Spectrometry and Tandem Mass Spectrometry. Analy. Biochem. 223:15-30.

Kemp, H.A., et al., (1986) Studies on the deterimental effects of bivalent binding in a microtiter plate ELISA and possible remedies. J. Immunol. Methods 94:65-72.

Marrs, C.F., et al. (1990) Pili (fimbriae) of Branhamella species. Am.J.Med. 88 (Suppl. 5A):36S-40S.

Masoud, H., et al. (1994) Structural elucidation of the backbone oligosaccharide for for the lipopolysaccharide of Moraxella catarrhalis serotype A. Can. J. Chem. 72:1466-1477.

Masoud, H., et al. (1994) Characterization of the lipopolysaccharide of Moraxella catarrhalis. Structural analysis of the lipid A from M. catarrhalis serotype A lipopolysaccharide. Eur.J. Biochem. 220:209-216.

McLeod, D.T., et al., (1986) Increase in bronchopulmonary infection due to Branhamella catarrhalis. Br. Med. J. 292:1103-1105.

Murphy, T.F. (1996) Branhamella catarrhalis: epidemiology, surface antigenic structure, and immune response. Microbiol. Rev. 60:267-279.

Murphy, T.F., et al. (1993) The major heat-modifiable outer membrane protein CD is highly conserved among strains of Branhamella catarrhalis. Mol. Microbiol. 10:87-97. 97.

Nicotra, B., et al. (1986) Branhamella catarrhalis as a lower respiratory tract

pathogen in patients with chronic lung disease. Arch. Intern. Med. 146:890-893.
Rahman, M., et al. (1995) Lack of serotype-specific antibody response to lipopolysaccharide antigens of Moraxella catarrhalis during lower respiratory tract infection. Eur. J. Clin. Microbiol. Infect. Dis. 14:297-304.
Rahman, M., et al. (1997) Human immunoglobulin isotype and IgG subclass response to different antigens of Moraxella catarrhalis. APMIS 105:213-220.
Robbins, J.B., et al. (1990) Polysaccharide-protein conjugates: a new generation of vaccines. J.Infect. Dis. 161:821-832.
Robbins, J.B., et al. (1995) Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J.Infect. Dis. 171:1387-1398.
Sarubbi, F.A., et al. (1990) Respiratory infections caused by Branhamella catarrhalis. Selected epidemiologic features. Am. J. Med. 88 Suppl 5A:9S-14S.
Smith, P.K., et al. (1985) Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76-85.
Svenson, S.B., et al. (1981) Artificial Samonella vaccines: Salmonella typhimurium O-antigen-specific oligosaccharide-protein conjugates elicit protective antibodies in rabbits and mice. Infect.Immun. 32:490-496.
Tsai, C.M., et al. (1982) A sensitive silver stain for detecting lipopolysaccharides lipopolysaccharides in polyacrylamide gels. Anal Biochem. 119:155-119.
Vaneechoutee, M., et al. (1990) Respiratory tract carrier rates of Moraxella (Branhamella) catarrhalis in adults and children and interpretation of the isolation isolation of M. catarrhalis from sputum. J. Clin. Microbiol. 28:2674-2680.
Vaneechoutee, M., et al. (1990) Serological Typing of Branhamella catarrhalis strains on the basis of lipopolysaccharide antigens. J. Clin. Microbiol. 28:182-187. 187.
Verheul, A.F.M., et al. (1991) Preparation, characterization, and immunogenicity of meningococcal immunotype L2 and L3,7,9, phosphoethanolamide group-containing oligosaccharide-protein conjugates. Infect.Immun. 59:843-851.
Wagner, D.K., et al. (1987) Analysis of immunoglobulin G antibody responses after administration of live and inactively influenza A vaccine indicates that nasal wash immunoglobulin G is a transudate from serum, J. Clin. Microbiol. 25:559-562.
W.H.O. Expert Committee on Biological Standardization (1991) Requirements for Haemophilus type b conjugate vaccines. WHO Tech. Rep. Ser. 814:15-37.
Yang, Y.P., et al. (1997) The major outer membrane protein, CD, extracted from Moraxella (Branhamella) catarrhalis is a potential vaccine antigen that induces bactericidal antibodies. FEMS Immunol. Med. Microbiol. 17:187-199.
Zollinger, W.D., et al. (1983) Importance of complement source in bactericidal activity of human and murine monoclonal antibody to meningococcal group B polysaccharide. Infect. Immun. 40:257-264.

ART-UNIT: 1645

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ATTY-AGENT-FIRM: Knobbe, Martens, Olson & Bear LLP

ABSTRACT:

A conjugate vaccine for Moraxella (Branhamella) catarrhalis comprising isolated lipooligosaccharide from which esterified fatty acids have been removed, to produce a detoxified lipooligosaccharide (dLOS), or from which lipid A has been removed, to produce a detoxified oligosaccharide (OS), which is linked to an immunogenic carrier. The vaccine is useful for preventing otitis media and respiratory infections caused by M. catarrhalis in mammals, including humans.

12 Claims, 3 Drawing figures

NeuNac polysaccharides, antibodies reactive with the capsular polysaccharide surface antigens of microorganisms containing poly α(2→9)NeuNac polysaccharide, and antibodies reactive with a carrier protein, comprising administering to said mammal a conjugate comprising *E. coli* K92 capsular polysaccharide linked to a carrier protein, under conditions suitable for eliciting said anti-polysaccharide and anti-carrier protein antibodies wherein said conjugate is the conjugate of claim **1**, claim **5**.

15. The method according to claim **14**, wherein said microorgansims containing poly α(2→8)NeuNAc polysaccharides are *Escherichia coli* K1 and *Neissertiae meningitidis* Group B, and wherein said microorganisms containing poly α(2→9)NeuNAc polysaccharide are *Neisseriae meningitidis* Group C.

16. An immunogenic preparation comprising the conjugate according to claim **1**, claim **5**, or claim **7** in a pharmaceutically-acceptable carrier, diluent, adjuvant, excipient, or mixtures thereof.

17. The preparation according to claim **16**, wherein said carrier protein is immunogenic, elicits a booster response, and confers said immunogenicity and said booster response to said conjugate.

18. A vaccine comprising the polysaccharide and carrier protein conjugate according to claim **1**, claim **5**, or claim **7**, said vaccine capable of being administered in an amount effective to elicit in a mammal antibodies reactive with poly α(2→8)NeuNAc capsular polysaccharides and with poly α(2→9)NeuNAc capsular polysaccharides of bacterial microorganisms.

19. The vaccine according to claim **18**, wherein said carrier protein is immunogenic, elicits a booster response, and confers said immunogenicity and said booster response to said conjugate.

20. The vaccine according to claim **19**, wherein said carrier protein is selected from the group consisting of albumins, chemically or genetically detoxified diphtheria toxin, tetanus toxoid, detoxified exotoxin A of *Pseudomonas aeruginosa,* detoxified *Staphylococcus aureus* toxin, synthetic polypeptides, bacterial outer membrane proteins, and viral proteins.

21. The vaccine according to claim **20**, wherein said carrier protein is tetanus toxoid.

22. Antibodies raised against said polysaccharide-protein conjugates according to claim **1**, claim **5**, or claim **7**.

23. The antibodies according to claim **22**, wherein said antibodies are selected from the group consisting of the IgM and IgG classes.

24. A method of passively immunizing a mammal to protect against infection from microorganisms containing poly α(2→8)NeuNAc capsular polysaccharides and with microorganisms containing poly α(2→9)NeuNAc capsular polysaccharides, comprising:

a) providing a conjugate comprising *E. coli* K92 capsular polysaccharide linked to a carrier protein;

b) producing antibodies directed against said conjugate of step a); and

c) administering said antibodies of step b) to a mammal wherein said conjugate is the conjugate of claim **1**, claim **5**.

25. A pharmaceutical composition comprising said polysaccharide-protein conjugate according to claim **1**, claim **5**, or claim **7** in a pharmaceutically acceptable carrier, excipient, or diluent.

26. The pharmaceutical composition according to claim **25**, wherein said composition is capable of being administered in an amount effective to elicit the production of IgM and IgG antibodies reactive with poly α(2→8)NeuNAc capsular polysaccharides of *Escherichia coli* K1 and Group B *Neisseriae meningitidis,* with poly α(2→9)NeuNAc capsular polysaccharide of Group C *Neisseriae meningitidis,* and with said carrier protein.

27. A method of producing a polysaccharide-carrier protein conjugate, said conjugate effective for eliciting serum IgM and IgG antibodies immunoreactive with poly α(2→8) NeuNAc-containing polysaccharides of *Escheilchia coli* K1 and Group B *Neisseriae meningitidis,* with poly α(2→8) NeuNAc-containing capsular polysaccharide of Group C meningococci, and with said carrier protein, wherein said polysaccharide of the conjugate is the capsular polysaccharide of *E. coli* K92, comprising the steps of:

a) derivatizing said K92 polysaccharide with a linker; and

b) covalently binding said derivatized polysaccharide of step a) to said carrier protein to form said immunoreactive conjugate.

28. The method according to claim **27**, wherein said linker is selected from the group consisting of adipic acid dihydrazide, diaminohexane, amino epsilon caproic acid, and N-hydroxysccinimide acid anhydride-based heterobifunctional molecules.

29. The method according to claim **28**, wherein said linker is adipic acid dihydrazide.

30. The method according to claim **28**, wherein said carrier protein of step b) is selected from the group consisting of albumins, chemically or genetically detoxified diphtheria toxin, tetanus toxoid, pertussis toxoid, detoxified exotoxin A of *Pseudomonas aeruginosa* detoxified *Staphylococcus aureus* toxin, synthetic polypeptides, bacterial outer membrane proteins, and viral proteins.

31. The method according to claim **30**, wherein said said said carrier protein is tetanus toxoid.

32. The conjugate according to claim **1**, wherein said diamino linker compound is diamino hexane.

33. The conjugate according to claim **1**, wherein said N-hydroxysuccinimide acid anhydride-based heterobifunctional molecule is N-succinimidyl 3-(2-pyridyldithio) proprionate.

34. The conjugate according to claim **5**, wherein said N-hydroxysuccinimide acid anhydride-based heterobifunctional molecule is N-succinimidyl 3-(2-pyridyldithio) proprionate.

35. The conjugate according to claim **7**, wherein said N-hydroxysuccinimide acid anhydride-based heterobifunctional molecule is N-succinimidyl 3-(2-pyridyldithio) proprionate.

36. The method according to claim **28**, wherein said diamino compound is diaminohexane.

37. The method according to claim **28**, wherein said N-hydroxysuccinimide acid anhydride-based heterobifunctional molecule is N-succinimidyl 3-(2-pyridyldithio) proprionate.

38. A method of producing a polysaccharide-carrier protein conjugate, said conjugate effective for eliciting serum IgM and IgG antibodies immunoreactive with poly α(2→8) NeuNAc-containing polysaccharides of *Escherichia coli* K1 and Group B *Neisseriae meningitidis,* with poly α(2→8) NeuNAc-containing capsular polysaccharide of Group C meningococci, and with said carrier protein, wherein said polysaccharide of the conjugate is the capsular polysaccharide of *E. coli* K92, comprising the steps of:

a) derivatizing said K92 polysaccharide with a linker selected from the group consisting of adipic acid dihydrazide or diamino hexane, amino epsilon caproic acid, and N-hydroxysuccinimide acid anhydride-based heterobifunctional molecules; and

b) covalently binding said derivatized polysaccharide of step a) to said carrier protein to form said immunoreactive antibody-eliciting conjugate.

* * * * *

US006632437B1

(12) **United States Patent**
Schneerson et al.

(10) **Patent No.: US 6,632,437 B1**
(45) **Date of Patent: Oct. 14, 2003**

(54) **IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES CONTAINING POLY α(2→8), α(2→9) NEUNAC CAPSULAR POLYSACCHARIDES**

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(73) Assignee: **The United States of America as represented by the Department of Health and Human Services**, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/153,263**

(22) Filed: **Nov. 15, 1993**

Related U.S. Application Data

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(51) **Int. Cl.**[7] **A61K 39/385**; A61K 39/395; A61K 39/108; C08H 1/00

(52) **U.S. Cl.** **424/193.1**; 424/137.1; 424/194.1; 424/197.11; 424/241.1; 530/403; 530/404; 530/405; 530/406

(58) **Field of Search** 424/193.1, 194.1, 424/197.11, 241.1, 137.1; 530/403–406

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,356,170	A	* 10/1982	Jennings et al.	424/92
4,619,828	A	* 10/1986	Gordon	424/92
4,695,624	A	* 9/1987	Marburg et al.	530/395
4,711,779	A	* 12/1987	Porro et al.	424/92
5,034,516	A	* 7/1991	Roy et al.	536/4.1

OTHER PUBLICATIONS

Osband et al (1990) Immunology Today 11(6):193–5.*
Gould et al (1989) J. Natl Cancer Inst. 81(10) 775–81.*
Dictionary of Immunology, 3rd ed, ed by WJ Herbert et al Blackwell Scientific Publications, Oxford 1985 pp. 65–66
Aldrich Catalog 1992–1993 p. 33 product No. 21782–4 Adipic dihydrafide.*
Häyrinen et al (1989) Molecular Immunology 26(6): 523–529.*
Chu et al (1983) Infection and Immunity 40(1):245–256.*
Bitter–Suermann et al (1987) Immunol. Res. 6:225–237.*
"Availability of Meningococcal Vaccine in Single–Dose Vitals for Travelers and High–Risk Persons", Morbidity and Mortality Weekly Report, Mass. Medical Society, vol. 39, No. 2, Oct. 26, 1990 p. ?.
Schlech, et al. "Bacterial Meningitis and Meningococcemia—United States, 1978", Morbidity and Mortality Weekly Report, Jun. 22, 1979, vol. 28, No. 24 pp 277–279.
Poolman et al., "Meningococcal Serotypes and Serogroup B Disease in North–West Europe", The Lancet, Sep. 6, 1986 pp. 555–557.
Brandtzaeg et al., "Plasma Endotoxin as a Predictor of Multiple Organ Failure and Death in Systemic Meningococcal Disease", The Journal of Infectious Diseases, vol. 159, No. 2, Feb. 1989, pp. 195–204.
Mendelman et al., "Genetic Diversity of Penicillin G–Resistant Neisseria Meningitidis from Spain", Infection and Immunity, vol. 57, No. 4, Apr. 1989, pp. 1025–1029.
Jennings et al., "Immunochemistry of Groups A, B, and C Meningococcal Polysaccaride–Tetanus Toxoid Conjugates", The Journal of Immunology, vol. 127, No. 3, Sep. 1981 pp 1011–1018.
Jennings et al., "N–Propionylated Group B Meningococcal Polysaccharide Mimics a Unique Epitope on Group B Neisseria Meningitidis", J. Exp. Med. vol. 165, Apr. 1987, pp. 1207–1211.
Frasch et al., "Antibody Response of Adults to an Aluminum Hydroxide–Adsorbed Neisseria Meningtidis Serotype 2b Protein–Group B Polysaccharide Vaccine", Journal of Infectious Diseases, vol. 158, No. 4, Oct. 1988 pp. 710–718.
Lifely, et al., "Immunogenicity in Adult Males of a Neisseria Meningitidis Group B Vaccine Composed of Poly–Saccharide Complexed with Outer Membrane Proteins", 60 Vaccine, vol. 9, Jan. 1991 pp. 60–66.
Zollinger et al., "Safety and Immunogenicity of a Neisseria Meningitidis Type 2 Protein Vaccine in Animals and Humans", The Journal of Infectious Diseases, vol. 137, No. 6, Jun. 1978 pp. 728–739.
Jennings, et al., "Conjugation of Meningococcal Lipopolysaccharide R–Type Oligosaccharides to Tetanus Toxoid as Route to a Potential Vaccine Against Group B Neisseria Meningitidis", Infection and Immunity, Jan. 1984, vol. 43, No. 1, pp. 407–412.
Verheul et al., "Preparation, Characterization and Immunogenicity of Meningococcal Immunotype L2 and L3,7,9, Phosphoethanolamine Group–Containing Oligosaccharide–Protein Conjugates", Infection and Immunity, vol. 59, No. 3, Mar. 1991, pp. 843–851.
Adlam et al., "Production of Colominic Acid by Pasteurella Haemolytica Serotype A2 Organisms", FEMS Microbiology Letters 42 (1987), pp. 23–25.
Frank, "The Role of Pasteurella Haemolytica in the Bovine Respiratory Disease Complex", Veterinary Medicine, Sep. 1986, pp. 838–846.
Frank, "Serotypes of Pasteurella Haemolytica in Sheep in the Midwestern United States", Amer. Journal of Veterinary Res., vol. 43, No. 11, pp. 2035–2037 1982.

(List continued on next page.)

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(74) *Attorney, Agent, or Firm*—Birch, Stewart, Kolasch & Birch, LLP

(57) **ABSTRACT**

The present invention relates to a polysaccharide-protein conjugate. The invention also relates to a method of using the conjugate to prevent systemic infections. The invention further relates to a pharmaceutical composition. The invention also relates to a method of producing a polysaccharide-protein conjugate.

38 Claims, 3 Drawing Sheets

OTHER PUBLICATIONS

Fraser et al., "Prevalence of Pasteurella Haemolytica Serotypes Isolated from Ovine Pasteurellosis in Britain", Veterinary Record, (1982), 110: 560–561.

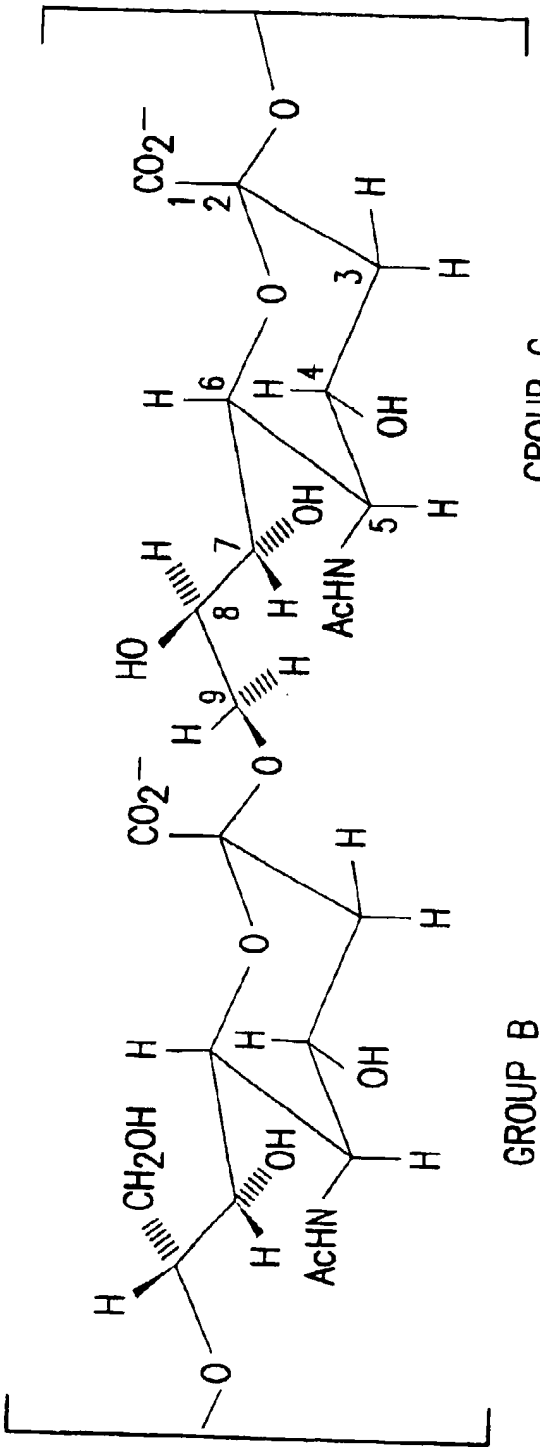
Gilmour et al., "The Development of Vaccines Against Pneumonic Pasteurellosis in Sheep", The Veterinary Record, Jan. 6, 1979, p. 15.

Thompson et al., "Serotypes of Pasteurella Haemolytica in Ovine Pasteurellosis", Research in Veterinary Science 1977, 22, pp. 130–131.

Jennings et al., "Induction of Meningococcal Group B Polysaccharide–Specific IgG Antibodies in Mice by Using an N–Propionylated B Polysaccharide–Tetanus Toxoid Conjugate Vaccine", Journal of Immunology, vol. 137, Sep. 1, 1986, pp. 1708–1713.

Moreno et al., "Immunity and Projection of Mice Against Neisseria Meningitidis Group B by Vaccination, Using Polysaccharide Complexed with Outer Membrane Proteins: A Comparision with Purified B Polysaccharide", Infection and Immunity, vol. 47, No. 2, Feb. 1985, pp. 527–533.

* cited by examiner



9)-NeuNAc-α-(2→8)-NeuNAc-α-(2→)

FIG. 1

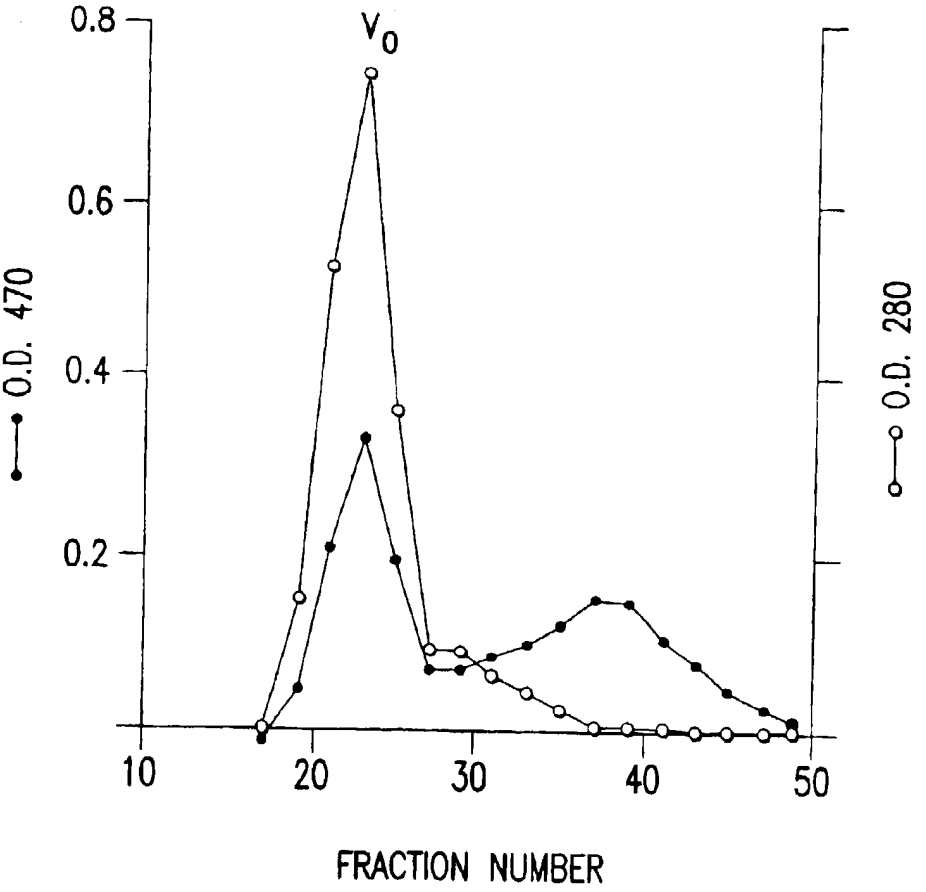


FIG.2

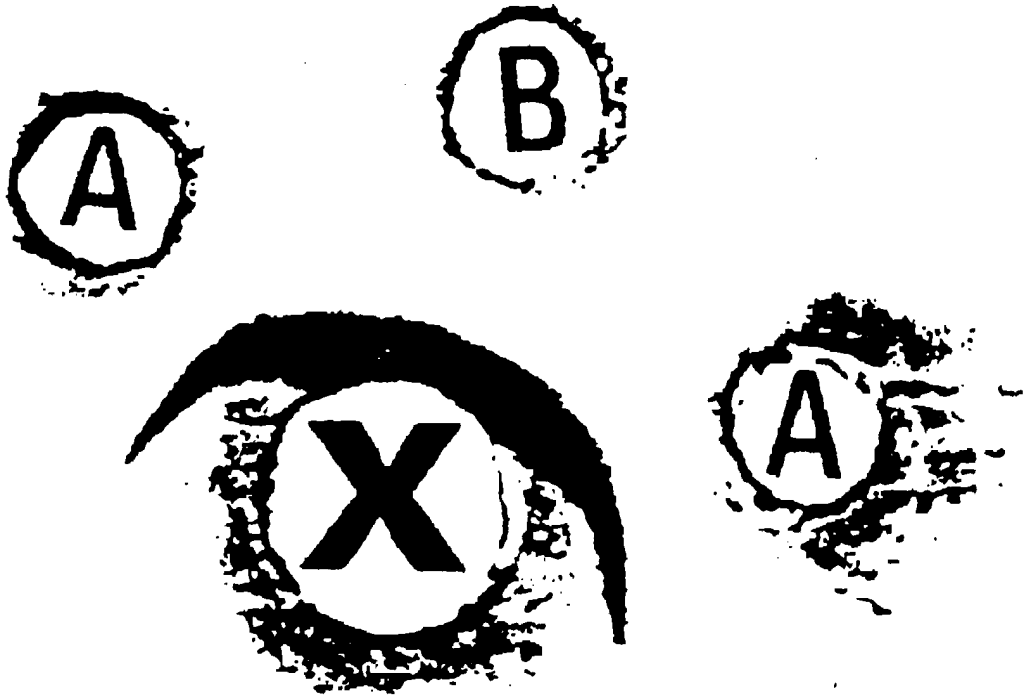


FIG.3

IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES CONTAINING POLY α(2→8), α(2→9) NEUNAC CAPSULAR POLYSACCHARIDES

This is a continuation of co-pending application Ser. No. 07/667,170, filed on Mar. 12, 1991, abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates, in general, to polysaccharide-protein conjugates and vaccines. In particular the present invention relates to polysaccharide-protein conjugates that elicit serum IgG and IgM antibodies to poly α(2→8) NeuNAc, or to both poly α(2→8) NeuNAc and poly α(2→9) NeuNAc, or to poly α(2→8),α(2→9) NeuNAc.

2. Background Information

Neisseriae meningitidis are a major cause of systemic infections, especially meningitis, in humans. Capsular polysaccharide (CP) vaccines are licensed for meningococcal groups A,C,Y and W135. Diseases caused by group B meningococci continue to occur in endemic and epidemic forms and remain an important health problem (Gotschlich, E. C. (1984) in Bacterial Vaccines. Ed. Germanier (Academic Press, NY) pp. 237–255; Peltola, H. (1983) Rev. Infect. Dis. 5, 71–91; Poolman, J. T. et al. (1986) Lancet, ii,555–557). *Escherichia coli* (*E. coli*) K1 is a major cause of neonatal meningitis, upper urinary tract infections and systemic infections in hospitalized patients and in domesticated and laboratory animals (Robbins, J. B. et al. (1974) N. Eng. J. Med. 290, 1216–1220; Kaijser, B. et al. (1977) Lancet i, 663–664; Cross, A. S. et al. (1984) J. Infect. Dis. 149, 184–193; Ørskov, I., & Ørskov, F. (1985) J. Hyg. Camb. 95, 551–575). Despite antibiotic treatment and supportive care, meningitis caused by these two pathogens continues to exert a high morbidity, including permanent CNS injury, and mortality (Peltola, H. (1983) Rev. Infect. Dis. 5, 71–91; Schneerson, R. (1988) in Understanding Mental Retardation. ed. Kavanagh, J. F. (Paul Brookes Publishing Co. Baltimore), pp. 237–249; Brandtzaeg, P. et al. (1989) J. Infect. Dis. 159, 195–204; McCracken, G. H., Jr. et al. (1974) Lancet, ii, 246–250).

The CP of Group B meningococci and of *E. coli* K1 are identical (poly α(2→8) NeuNAc) and serve as essential virulence factors and protective antigens for both pathogens (Grados, O., & Ewing, W. H. (1970) J. Infect. Dis. 122, 100–103; Kasper, D. L. et al. (1973) J. Immunol. 110, 262–268; Bhattacharjee, A. K. et al. (1975) J. Biol. Chem. 250, 1926–1932; Robbins, J. B. et al. (1974) N. Eng. J. Med. 290, 1216–1220). Poly α(2→8) NeuNAc is also a surface antigen of *Moraxella nonliquefaciens* and *Pasteurella haemolytica,* serotype A-2 (Bøvre, K. et al. (1983) NIHP Annals. 6, 65–73; Devi, S. J. N. et al. (1991) Infect. Immun. 59, 732–736; Adlam, C. et al. (1987) FEMS Microbiol. Lett. 42, 23–25). The latter is the major cause of outbreaks of pasteurellosis in young lambs which suggests that poly α(2→8) NeuNAc may serve as a virulence factor for yet another bacterial species.

Attempts to induce protective immunity to group B meningococci and *E. coli* K1 have been thwarted because poly α(2→8) NeuNAc, alone or complexed to outer membrane proteins, induced low and transient levels of IgM antibodies (Kasper, D. L. et al. (1973) J. Immunol. 110, 262–268; Wyle, F. A. et al. (1972) J. Infect. Dis. 126, 514–522; Zollinger, W. D. et al. (1979) J. Clin. Invest. 63, 836–842; Moreno, C. et al. (1985) Infect. Immun. 47, 527–533; Frasch, C. E. et al. (1988) J. Infect. Dis. 158, 710–718; Lifely, M. R. et al. (1991) Vaccine 9, 60–66). Covalent attachment of periodate-treated (Jennings, H. & Lugowski, C. (1981) J. Immunol. 127, 1011–1018) or acid-hydrolyzed poly α(2→8) NeuNAc (Porro, M. et al. (1983) Med. Trop. 43, 129–132) to a protein also failed to elicit antibodies to this antigen. Further, this CP has been considered as a "self antigen", because α(2→8) NeuNAc is found as monomers or dimers on glycoproteins and gangliosides in adults and up to ≈11 residues in fetal tissues including N-CAMs (Finne, J. et al. (1983) Lancet, ii, 355–357; Finne, J. et al. (1987) J. Immunol. 138, 4402–4407; Soderstrom, T. et al. (1984) N. Eng. J. Med. 310, 726–727). Accordingly, investigators have studied other components, such as LPS, outer membrane proteins and iron-binding proteins, or chemically modified poly α(2→8) NeuNAc, as potential vaccines (Zollinger, W. D. et al. (1979) J. Clin. Invest. 63, 836–842; Moreno, C. et al. (1985) Infect. Immun. 47, 527–533; Frasch, C. E. et al. (1988) J. Infect. Dis. 158, 710–718; Jennings, H. J. et al. (1984) Infect. Immun. 43, 407–412; Jennings, H. J. et al. (1986) J. Immunol. 137, 1708–1713; Frasch, C. E. (1989) Clin. Microbiol. Rev. 2(Suppl), S134-S138).

Most newborns and adults have bactericidal antibodies to the three major serogroups (A,B,C) of meningococci (Goldschneider, I. et al. (1969) J. Exp. Med. 129, 1307–1326); most of the bactericidal activity, including of group B meningococci, was removed by adsorption with the homologous CP (Frasch, C. E. et al. (1988) J. Infect. Dis. 158, 710–718; Brandt, B. L. et al. (1972) J. Immunol. 108, 913–920; Kasper, D. L. et al. (1973) J. Infect. Dis. 127, 378–387; Skevakis, L. et al. (1984) J. Infect. Dis. 149, 387–396). The peak incidence of disease caused by meningococci, including group B, is when the maternally-derived antibodies have waned and the adult levels have not yet developed (Gotschlich, E. C. (1984) in Bacterial Vaccines. Ed. Germanier (Academic Press, NY) pp. 237–255; Goldschneider, I. et al. (1969) J. Exp. Med. 129, 1307–1326). Rises in poly αa(2→8) NeuNAc antibodies, including those of the IgG isotype, are detectable in patients convalescent from group B meningococcal meningitis (Wyle, F. A. et al. (1972) J. Infect. Dis. 126, 514–522; Zollinger, W. D. et al. (1979) J. Clin. Invest. 63, 836–842; Frasch, C. E. et al. (1988) J. Infect. Dis. 158, 710–718; Skevakis, L. et al. (1984) J. Infect. Dis. 149, 387–396; Craven, D. E. et al. (1982) Infect. Immun. 37, 132–137; Mandrell, R. E. & Zollinger, W. D. (1982) J. Immunol. 129, 2172–2178; Leinonen, M. & Frasch, C. E. (1982) Infect. Immun. 38, 1203–1207). Polyclonal and monoclonal (mAb) poly α(2→8) NeuNAc antibodies were raised in animals by multiple intravenous injections of bacteria (Robbins, J. B. et al. (1974) N. Eng. J. Med. 290, 1216–1220; Moreno, C. et al. (1985) Infect. Immun. 47, 527–533; Mandrell, R. E. & Zollinger, W. D. (1982) J. Immunol. 129, 2172–2178; Allen, P. Z. et al. (1982) J. Clin. Microbiol. 15, 324–329; Craven, D. E. et al. (1979) J. Clin. Microbiol. 10, 302–307; Frosch, M. et al. (1985) Proc. Natl. Acad. Sci. (USA) 82, 1194–1198). Monoclonal antibodies to this antigen were identified in a healthy 81 year old male and from hybridoma cultures (Kabat, E. A. et al. (1986) J. Exp. Med. 164, 642–654; Kabat, E. A. et al. (1988) J. Exp. Med. 168, 699–711; Raff, H. V. et al. (1988) J. Infect. Dis. 157, 118–126). These antibodies exert biologic activities which have been correlated with protective immunity; 1) complement-dependent bacteriolysis on Group B meningococci (Gotschlich, E. C. (1984) in Bacterial Vaccines. Ed. Germanier (Academic Press, NY) pp. 237–255;

Goldschneider, I. et al. (1969) J. Exp. Med. 129, 1307–1326); 2) protection against lethal infection of rodents by *E. coli* K1 (Robbins, J. B. et al. (1974) N. Eng. J. Med. 290, 1216–1220; Glode, M. P. et al. (1977) Infect. Immun. 16, 75–80; Kim, K. S. et al. (1985) Infect. Immun. 50, 734–737).

There are two other bacterial NeuNAc polymers: 1) group C *N. meningitidis* CP composed of poly α(2→9) NeuNAc; most strains are variably O-acetylated at C7 or C8 (Bhattacharjee, A. K. et al. (1975) J. Biol. Chem. 250, 1926–1932). Although differing from poly α(2→8) NeuNAc only by linkage, poly α(2→9) NeuNAc is immunogenic and is a licensed vaccine against group C meningococci (World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland); 2) *E. coli* K92 CP (FIG. **1**) with the disaccharide repeat unit of alternating α(2→8),α(2→9) NeuNAc (The structure of this polysaccharide can be written as 9)-NeuNAc-α-(2→8)-NeuNAc-α-(2→.) (Robbins, J. B. et al. (1972) Infect. Immun. 6, 651–656; Glode, M. P. et al. (1977) J. Infect. Dis. 135, 94–102; Egan, W. et al. (1977) Biochem. (USA) 16, 3687–3692; Glode, M. P. et al. (1979) J. Infect. Dis. 139, 52–59). Both group B and group C meningococcal antisera precipitate with *E. coli* K92 CP (Glode, M. P. et al. (1977) J. Infect. Dis. 135, 94–102; Egan, W. et al. (1977) Biochem. (USA) 16, 3687–3692; Glode, M. P. et al. (1979) J. Infect. Dis. 139, 52–59). Multiple i.v. injections of killed *E. coli* K92 bacteria induced precipitating antibodies to poly α(2→9) NeuNAc and to poly α(2→8),α(2→9) NeuNAc but not to poly α(2→8) NeuNAc (Glode, M. P. et al. (1977) J. Infect. Dis. 135, 94–102). Injection of *E. coli* K92 CP induced poly α(2→9) NeuNAc antibodies in adult volunteers; antibodies to poly α(2→8) NeuNAc were not measured (Glode, M. P. et al. (1979) J. Infect. Dis. 139, 52–59).

SUMMARY OF THE INVENTION

It is a general object of this invention to provide a polysaccharide-protein conjugate and a vaccine.

It is a specific object of this invention to provide a polysaccharide-protein conjugate capable of eliciting serum IgG and IgM antibodies to poly α(2→8) NeuNAc, or to both poly α(2→8) NeuNAc and poly α(2→9) NeuNAc, or to poly α(2→8),α(2→9) NeuNAc.

It is a further object of this invention to provide a pharmaceutical composition suitable for use in preventing systemic infections.

It is another object of this invention to provide a method of preventing systemic infections.

It is a further object of this invention to provide a method of preventing systemic infections caused by Groups A, B, and C *Neisseria meningitidis*.

It is another object of this invention to provide a method of producing a polysaccharide-protein conjugate.

Further objects and advantages of the present invention will be clear from the description that follows.

In one embodiment, the present invention relates to a polysaccharide-protein conjugate comprising a polysaccharide and a carrier protein wherein the conjugate is capable of eliciting serum IgG and IgM antibodies to poly α(2→8) NeuNAc, or to both poly α(2→8) NeuNAc and poly α(2→9) NeuNAc, or to poly α(2→8),α(2→9) NeuNAc in a mammal or bird.

In another embodiment, the present invention relates to a pharmaceutical composition and a vaccine comprising a polysaccharide-protein conjugate in an amount sufficient to prevent systemic infections, and a pharmaceutically acceptable diluent, carrier, or excipient.

In a further embodiment, the present invention relates to a method of preventing systemic infections in an animal comprising administering to the animal an amount of a polysaccharide-protein conjugate sufficient to effect the prevention.

In another embodiment, the present invention relates to a method of preventing systemic infections caused by Groups A,-B, and C *Neisseria meningitidis* in an animal comprising administering to the animal the above-described polysaccharide-protein conjugate and a Group A meningococcal polysaccharide-protein conjugate under conditions such that the infections are prevented.

In yet another embodiment, the present invention relates to a method of producing a polysaccharide-protein conjugate comprising derivatizing a polysaccharide and conjugating the derivatized polysaccharide to a protein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**. Structure of the *Escherichia coli* K92 capsular polysaccharide: a disaccharide repeat unit of alternating poly α(2→8),α(2→9) NeuNAc (Egan, W., et al. (1977) Biochem. (USA) 13, 3687–3692). The structure of this polysaccharide can be written as 9)-NeuNAc-α-(2→8)-NeuNAc-α-(2α).

FIG. **2**. Gel filtration of K92-TT_1(tetanus toxoid) conjugate. 1.0 ml of K92-TT_1, was passed through a column of 4B-CL Sepharose (2.5×90 cm) in 0.2M NaCl. The fraction size was 2.0 ml and the eluent was monitored by assay of NeuNAc (Yao, K. & Ubuka, T. (1987) Acta Med. Okayama. 41, 237–241) and by absorbance at 280 nm (World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland; Schneerson, R. et al. (1980) J. Exp. Med. 152, 361–376).

FIG. **3**. Double immunodiffusion with K92 conjugate: Center well—K92-TT_1, 0.1 mg/ml, Well A—rabbit antiserum to *Escherichia coli* K92 cells, Well B—mouse tetanus toxin antiserum.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a polysaccharide-protein conjugate and a vaccine. This conjugate includes a polysaccharide and a carrier protein and is capable of eliciting serum IgG and IgM antibodies to poly α(2→8) NeuNAc, or to both poly α(2→8) NeuNAc and poly α(2→9) NeuNAc, or to poly α(2→8),α(2→9) NeuNAc in a mammal or bird. The carrier is associated with the polysaccharide in such a way as to increase the immunogenicity of the polysaccharide and to confer upon it the properties of both eliciting a booster response and IgG antibodies. These immunologic properties should be elicited by the protein-polysaccharide vaccine alone. Addition of adjuvants, such as aluminum salts, bacterial murein structures in saline or in emulsions, may be helpful in eliciting or in enhancing the production of poly α(2→8) NeuNAc and poly α(2→9) NeuNAc Antibodies by the *E. coli* K92 and the poly α(2→8) NeuNAc conjugate vaccines. In one preferred embodiment, the carrier protein is covalently bound to the polysaccharide. The covalent bond should preserve the immunologic properties of the native polysaccharide and native protein. Some proteins that could serve as effective carriers for covalently bound polysaccharide-protein conjugates are albumins, pharmaco-

logically active proteins that have been detoxified, by chemical or genetic mechanisms, including diptheria, tetanus, pertussis, *Pseudomonas aeruginosa* exotoxin A and *Stapylococcus aureus* toxins, synthetic polypeptides, bacterial outer membrane proteins and viral proteins (Schneerson, R. et al. (1980) In: New Developments with Human and Veterinary Vaccines. Eds. Mizrahi et al., New York, Alan R. Liss; Schneerson, R. et al. (1987) In: Towards Better Carbohydrate Vaccines. Eds., Bell, R. & Torrigiani, G., World Health Organization, John Wiley & Sons, Ltd.). Carriers for the K92 or the poly α(2→8) NeuNAc polysaccharides should be proteins that are immunogenic and elicit booster responses by themselves. Carriers should have the necessary groups that allow the synthesis of conjugates with the *E. coli* K92 or poly α(2→8) NeuNAc polysaccharides. Carriers should confer the properties of increased immunogenicity and booster responses to the *E. coli* K92 and poly α(2→8) NeuNAc including the formation of both IgM and IgG antibodies to these polysaccharides (Schneerson et al (1987) In: Towards Better Carbohydrate Vaccines. Eds., Bell, R. & Torrigiani, G., World Health Organization, John Wiley & Sons, Ltd.). In another preferred embodiment, the polysaccharide and protein are covalently bound by a linker. An effective linker has been found to be adipic acid dihydrazide. Other linkers could be diaminohexane, amino epsilon caproic acid, N-hydroxysuccinimide acid anhydride based heterobifunctional linkers as illustrated by N-succinimidyl 3-(2-pyridyldithio)priopionate (SPDP). Other cross-linking compounds can be used to synthesize the conjugate, provided they are not toxic and result in a conjugate that elicits poly α(2→8) NeuNAc and poly α(2→9) NeuNAc antibodies (Robbins, J. B. & Schneerson, R. (1990) J. Infect. Dis. 161:821–832). A linker is a molecule which may be used to covalently bind the polysaccharide to the protein. A chemical reaction with each end of the linker changes the structure of the linker. For example, after adipic acid dihydrazide chemically combines with the polysaccharide and the protein to form a conjugate, the polysaccharide and protein are bound by an adipic acid dihydrazido linkage. In another preferred embodiment, the polysaccharide comprises poly α(2→8) NeuNAc or derivatives thereof. In a further preferred embodiment, the polysaccharide comprises a heteropolymer of α(2→8),α(2→9) NeuNAc or derivatives thereof. In yet another preferred embodiment, the carrier protein is tetanus toxoid. Additional carrier proteins that may be used include albumins (Schneerson, R., et al. (1980) J. Exp. Med. 152, 361–376), diphtheria toxoid (Schneerson, R., et al. (1980) J. Exp. Med. 152, 361–376), and *Pseudomonas aeruginosa* exotoxin A and mutants of this protein (Fattom, A., et al. (1990) Infect. Immun. 58, 2367–2374).

In another embodiment, the present invention relates to a pharmaceutical composition comprising the above described polysaccharide-protein conjugate in an amount sufficient to prevent systemic infections including meningitis, caused by group B or group C *Neisseria meningitidis, Escherichia coli* K1, *Moraxella nonliquefaciens, Pasteurella haemolytica,* or other microorganisms containing poly α(2→8) NeuNAc, poly α(2→9) NeuNAc, or poly α(2→8),α(2→9) NeuNAc surface antigens and a pharmaceutically acceptable diluent, carrier, or excipient. The pharmaceutical composition of the invention includes polysaccharide conjugate in a quantity selected depending on the route of administration. Although subcutaneous or intramuscular routes of administration are preferred, the above described polysaccharide-protein conjugate could also be administered by an intraperitoneal or intravenous route. One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can be readily determined. Suitable amounts might be expected to fall within the range of 5.0 micrograms per dose to 100.0 micrograms per dose of either the polysaccharide or the protein (The ratios of polysaccharide and protein that comprise the conjugate may differ. The dosages mentioned for each component are within the expected range.).

In another embodiment, the present invention relates to a method of using the above described polysaccharide-protein conjugate to prevent the above described systemic infections. One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can readily be determined.

In yet another embodiment, the present invention relates to a method of preventing systemic infections caused by Groups A, B, and C *Neisseria meningitidis* in an animal comprising administering to the animal the above-described polysaccharide-protein conjugate and a Group A meningococcal polysaccharide-protein conjugate under conditions such that the infections are prevented. The compositions also serve as vaccines.

In another embodiment, the present invention relates to a method of producing a polysaccharide-protein conjugate effective in eliciting serum IgG and IgM antibodies to poly α(2→8) NeuNAc, or to both poly α(2→8) NeuNAc and poly α(2→9) NeuNAc, or to poly α(2→8),α(2→9) NeuNAc in a mammal or bird. The first step of the method comprises derivatizing the polysaccharide by using, for example, adipic acid dihydrazide in a carbodiimide reaction, or alternative agents/protocols. Adipic acid dihydrazide may be substituted in the carbodiimide reaction with other dihydrazide compounds or diamino compounds (for example: diamino hexane). Other derivatives of the polysaccharides could be made in order to covalently bind them to proteins. These include the use of disulfide bonds linked by heterobifunctional reagents (Szu, S. C., et al. (1986) Infect. Immun. 54, 448–455; Szu, S. C., et al. (1987) J. Exp. Med. 166, 1510–1524).

After derivatizing the polysaccharide, the next step of the method involves conjugating the derivative to a protein. Preferably, the adipic acid hydrazide derivative of the polysaccharide is conjugated to the protein by mixing the derivative with the carrier protein at equal concentrations and adjusting the pH to a pH in the range between 6.1 and 7.0. The reactants are dissolved in 0.2M NaCl and the temperature is at 3–8° C. Then, 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDAC) is added to a final concentration less than 0.3M. The original pH is maintained for 3 hours. Next, the reaction mixture is dialyzed against 0.2M NaCl at 3–8° C. for 3 days with multiple changes of the outer fluid. This synthetic scheme of multipoint attachment does not grossly fragment the poly α(2→8) NeuNAc or poly α(2→8),α(2→9) NeuNAc and may provide conformational stability to the polysaccharide.

The invention is described in further detail in the following non-limiting examples.

EXAMPLES

The following protocols and experimental details are referenced in the examples that follow:

Bacteria. *E. coli* 07:K1:H- strain C94, *E. coli* 016:K1:H-, stable in the O acetyl negative form (OAc^-), *E. coli* 075:K1:H-, OAc^+, strain LH, (Lars A. Hanson, Goteborg, Sweden), *E. coli* 013:K92:H4, strain N67 have been described (Robbins, J. B. et al. (1972) Infect. Immun. 6, 651–656). Group B meningococci, serotype 6, strain

M990 and strain B11, and Group C meningococcus, strain C11, were provided by Carl E. Frasch, FDA, Bethesda, Md.

Polysaccharides and proteins. CP were purified from Group B meningococcus, strains B11 and M990, *E. coli* strains C94, LH, 016:K1:H- and N67 (World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland). These CP contained <1.0% of protein and nucleic acid, 75 to 87% NeuNAc (Yao, K. & Ubuka, T. (1987) Acta Med. Okayama. 41, 237–241), <0.01% of LPS and had Kd values through 4B-CL Sepharose of ~0.5 (World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland). The OAc contents were 1.62 μM/mg for LH and 1.39 μM/mg for the group C meningococcal CP (Bhattacharjee, A. K. et al. (1975) J. Biol. Chem. 250, 1926–1932; World Health Organization Expert Committee on Biological Standardization. (1977) Technical Report Series, 610. WHO, Geneva, Switzerland). The ^{13}C and proton NMR spectra of the poly α(2→8) NeuNAc and K92 CP were identical to those reported for these two polymers (Bhattacharjee, A. K. et al. (1975) J. Biol. Chem. 250, 1926–1932; Egan, W. et al. (1977) Biochem. (USA) 16, 3687–3692). Group C meningococcal CP was obtained from Pat Mcverry, Connaught Laboratories Inc, Swiftwater, Pa., and tetanus toxoid (TT), lot GYA, and group A meningococcal CP from Dominique Schulz, Pasteur Merieux Serums and Vaccines, Lyon, France. Type III, group B streptococcus CP was purified in the laboratory (Lagergard, T. et al. (1990) Infect. & Immun. 58, 687–694).

Hyperimmune sera. Antisera, prepared by intravenous injections of killed cells of Group B meningococci, strain B11 (horse 46), Group C meningococci, strain C11 (burro 211) and rabbit *E. coli* K92 (Drs. Ida and Frits Ørskov, Statens Seruminstitut, Copenhagen, Denmark) have been described (Ørskov, I., & Ørskov, F. (1985) J. Hyg. Camb. 95, 551–575; Allen, P. Z. et al. (1982) J. Clin. Microbiol. 15, 324–329; Glode, M. P. et al. (1977) J. Infect. Dis. 135, 94–102; Ørskov F. et al. (1979) J. Exp. Med. 149, 669–685). Mice were injected with formalin-killed cells and their sera harvested as described (Ørskov, I., & Ørskov, F. (1985) J. Hyg. Camb. 95, 551–575; Lagergard, T. et al. (1990) Infect. & Immun. 58, 687–694). Antisera for standards were produced in NIH general purpose mice by i.p. injection of 5.0 μg of either TT or K1-TT_2 in Freund's adjuvants (Lagergard, T. et al. (1990) Infect. & Immun. 58, 687–694).

Serology. Double immunodiffusion was performed in 0.6% agarose. ELISA was performed using biotinylated CP (Sutton, A. et al. (1985) J. Immunol. Meth. 82, 215–224). Murine sera were assayed for poly α(2→8) NeuNAc and poly α(2→9) NeuNAc and TT antibodies using alkaline-phosphatase-labeled goat anti-murine immunoglobulins (Kirkgaard & Perry Inc, Gaithersburg, Md.) (Lagergard, T. et al. (1989) Infect. & Immun. 58, 687–694; Sutton, A. et al. (1985) J. Immunol. Meth. 82, 215–224). Murine IgM mAb to poly α(2→8) NeuNAc (Wendell Zollinger, Walter Reed Army Institute of Research, Washington, D.C.) and murine IgM and IgG mAb to poly α(2→9) NeuNAc (Kathryn Stein, FDA, Rockville, Md.) were used as reference standards (Mandrell, R. E. & Zollinger, W. D. (1982) J. Immunol. 129, 2172–2178; Rubinstein, L. J. & Stein, K. E. (1988) J. Immunol. 141, 4357–4362). Human poly α(2→8) NeuNAc antibodies were assayed as described (Claesson, B. O. et al. (1988) J. Pediatr. 112, 695–702). A human IgM mAb (Elvin Kabat, Columbia University, NY) (Kabat, E. A. et al. (1986) J. Exp. Med. 164, 642–654; Kabat, E. A. et al. (1988) J. Exp. Med. 168, 699–711) and a high-titered human serum (GH) were used as references for human poly α(2→8) NeuNAc antibodies and the data are expressed as μg/ml for the IgM and as percent of the standard for IgG.

The effect of temperature upon IgG binding to poly α(2→8) NeuNAc and poly α(2→9) NeuNAc was assayed with sera from mice injected with bacteria or three times with 10.0 μg of conjugates. The data are expressed as the percent binding at 37° C. compared to 22° C.

Synthesis of conjugates. It was confirmed that treatment at pH <6.0 or with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) at concentrations >0.3M, even at neutral pH, resulted in loss of antigenicity of poly α(2→8) NeuNAc (Lifely, M. R., Gilbert, A. S. & Moreno, C. (1981) Carb. Res. 94, 193–201). Accordingly, the CP (5.0 mg/ml in 0.2M NaCl) were derivatized with 0.5M adipic acid dihydrazide (ADH), 0.1M EDAC, pH 6.1 to 7.0 at room temperature for 3 to 4 h. The pH was maintained in a pH stat with 0.25N HCl. The reaction mixture was dialyzed against 0.2M NaCl at 3–8° C., for 2 days with 3 changes of the outer fluid and passed through 4B-CL Sepharose in this solvent. The CP-containing fractions were pooled, dialyzed against sterile pyrogen-free water and freeze-dried. The content of adipic acid hydrazide (AH) was assayed by the TNBS reaction (Inman, J. K., & H. M. Dintzis. (1969) Biochem. (USA) 8, 4074–4080; Schneerson, R. et al. (1980) J. Exp. Med. 152, 361–376). AH-CP and TT, at equal concentrations of 7.5 to 20 mg/ml in 0.2M NaCl, were adjusted to a pH between 6.1 and 7.0 with 0.1N HCl. Then, 0.1M EDAC was added and this pH maintained at 3–8° C. for 3 h. The reaction mixture was dialyzed against 0.2M NaCl at 3–8° C. and then passed through 4B-CL Sepharose in the same solvent. The void volume fractions were pooled, assayed for NeuNAc and protein and stored in 0.01% thimerosal at 3–8° C.

Immunization of mice. General purpose mice, 4 to 5 weeks old, were injected s.c. with 2.5 μg of NeuNAc in 0.1 ml of saline, either as the CP alone or as the conjugate, 3 times 2 weeks apart (Schneerson, R. et al. (1980) J. Exp. Med. 152, 361–376). Ten mice from each group were exsanguinated 7 days after each injection. None of the mice injected with saline (controls) had antibodies to the CP or to TT (data not shown).

Adsorption. ELISA was used to determine the specificity of IgG poly α(2→8) NeuNAc and poly α(2→9) NeuNAc antibodies. Dilutions of sera that yielded an A in the upper linear part of the curve (1.0 to 1.4) were mixed with 100 μg of either poly α(2→8) NeuNAc, poly α(2→9) NeuNAc, or K92 CP and incubated at 22° C. for 2 h and overnight at 3–8° C. Controls were the group A meningococcal and the type III group B streptococcal CP (containing an α(2→8)-linked NeuNAc residue per repeat unit). Adsorption by the CP was calculated as the percent A compared to the unadsorbed sera.

Human sera. Paired maternal and cord sera were donated by James C. Parke Jr, Charlotte Memorial Hospital and Medical Center, Charlotte, N.C. and Eyal Schiff and Justin Passwell, Sheba Medical Center, Israel.

Statistical methods. Data analysis was performed using the Statistical Analysis System (SAS). The logarithms of the concentrations were used for all statistical calculations. Antibody concentrations that were below the limit of sensitivity of the ELISA were assigned values equal to one half of that value. Comparison of geometric means was performed with the two-sided t-test and the paired t-test.

Example 1

Characterization of the Conjugates

Data of representative conjugates are shown in Table 1. The percent of derivatization of the CP with AH ranged from 0.8 for K1-TT_1 to 10.2 for K92-TT_2. All AH derivatives, except for the latter, yielded an identity reaction with the native CP by double immunodiffusion. The native CP formed a spur over this K92-AH derivative (not shown).

The protein/NeuNAc ratios were related to the percent derivatization of the CP with AH. K1-TT_1 had the highest protein/NeuNAc ratio (12.8) and contained a CP with 0.8% AH. K92-TT_2, with the lowest ratio (1.4), contained a CP with 10.2% AH. The highest yields of conjugates were obtained when the reaction mixture for conjugation used concentrations of 7.5 to 10 mg/ml of TT and AH-CP.

All preparations of conjugates eluted at the void volume through CL-4B Sepharose indicating multipoint attachment between the AH-CP and the TT (FIG. 2). FIG. 3 provides serologic evidence for the covalent attachment of the CP with the carrier protein (TT). Antiserum to each component precipitated with a line of identity with a representative conjugate, K1-TT_1. Non-identical lines of precipitation were formed when these antisera reacted with mixtures of the CP and TT (not shown).

TABLE 1

Characterization of capsular polysaccharide-protein conjugates

Conjugate	Protein (μg/ml)	NeuNAc (μg/ml)	AH/Neu-NAc (wt/wt)	Protein/CP ratio	Yield (% CP)	Concentration (mg/ml)*
K1-TT_1**	531	41.4	0.8	12.8	5.0	20
K1-TT_2**	465	96.1	2.6	4.8	9.6	15
$K1_{OAc+}$-TT***	630	262	1.9	2.4	28.8	10
MenB-TT_1	463	94.5	1.8	4.9	5.0	15
MenB-TT_2	314	51.1	2.3	6.2	4.6	15
K92-TT_1	294	98.8	3.4	3.0	10.5	15
K92-TT_2	705	517	10.2	1.4	51.7	7.5
MenC-TT	234	121	8.5	1.9	22.5	10

*Concentration of the reactants during the conjugation procedure.
**The K1 CP for these conjugates were OAc^-.
***K1 CP of the OAc^+ variant of *Escherichia coli*, strain LH.

Example 2

Induction of Poly α(2→8) NeuNAc Antibodies (Table 2)

The four CP did not elicit rises of IgM or IgG antibodies. All four poly α(2→8) NeuNAc conjugates (K1-TT_1, K1-TT_2, MenB-TT_1 and MenB-TT_2) elicited statistically significant rises in IgM antibodies. An IgM booster response was elicited after the second injection by these conjugates; the levels elicited by K1-TT_1 and MenB-TT_1 were higher than those elicited by the other two poly α(2→8) NeuNAc conjugates ($p<0.001$). Only K1-TT_2 and MenB-TT_2 elicited IgM booster responses after the third injection.

The four poly α(2→8) NeuNAc conjugates elicited statistically significant rises of IgG antibodies after the second and third injections. The IgG levels elicited by the third injection of MenB-TT_2 (4.29 U/ml) were higher than those elicited by the other three conjugates but not significant (NS). One mouse in this group, however, had 240 U/ml and the geometric mean level, excluding this animal, was 2.74 U/ml.

$K1_{OAc+}$-TT, prepared from *E. coli* strain LH, elicited high levels of IgM and IgG antibody to the OAc^+ variant of this CP and low antibody levels to poly α(2→8) NeuNAc.

The two K92-TT elicited both IgM and IgG poly α(2→8) NeuNAc antibodies; the IgG levels were higher than those elicited by the K1-TT_1 ($P=0.01$), K1-TT_2 ($P=0.0001$), MenB-TT_1 ($P=0.0002$) and MenB-TT2 ($p<0.05$). K92-TT2, containing the heavily derivatized K92 CP, also elicited higher IgG antibody levels than the K1-TT and MenB-TT conjugates.

MenC-TT did not elicit poly α(2→8) NeuNAc antibodies in any of the mice.

The specificity of the antibodies was shown by adsorption experiments using sera from mice injected with killed bacteria or by three injections of the conjugates (data not shown). Poly α(2→8) NeuNAc and poly α(2→9) NeuNAc adsorbed homologous IgG antibodies from the antisera (50–89%). The K92 CP adsorbed both poly α(2→8) and poly α(2→9) NeuNAc antibodies (69–89%). The two controls (group A meningococcal and group B type III streptococcal CP) adsorbed <10% of either poly α(2→8) or α(2→9) NeuNAc antibodies.

TABLE 2

Serum IgG and IgM antibodies to the capsular polysaccharide of Group B *Neisseria meningitidis* and *Escherichia coli* K1 (poly α(2→8) NeuNAc).

	Post-immunization geometric mean					
	IgM (μg/ml)			IgG (ELISA U)		
Immunogen	1st	2nd	3rd	1st	2nd	3rd
K1	0.09	0.12	0.11[a]	0.05	0.05	0.06[c]
K1-TT_1	0.32	3.35	1.63[b]	0.10	0.49	2.44[d]
K1-TT_2	0.12	0.19	0.62[b]	0.06	0.13	1.95[e]
$K1_{OAc+}$-TT*	0.17	0.16	0.08	0.07	0.20	0.72
	38.7	27.2	7.18	0.16	12.1	56.1
MenB	0.05	0.05	0.05[a]	0.05	0.05	0.05[c]
MenB-TT_1	0.67	1.59	1.50[b]	0.08	0.45	1.81[f]
MenB-TT_2	0.08	0.26	0.72[b]	0.05	0.11	4.29[g]
K92	0.05	0.05	0.05[a]	0.05	0.05	0.05[c]
K92-TT_1	0.09	0.49	1.20[b]	0.05	0.25	17.2[h]
K92-TT_2	0.28	0.78	0.47[b]	0.05	0.83	4.52[i]
MenC	0.05	0.05	0.05	0.05	0.05	0.05
MenC-TT	0.05	0.05	0.05	0.05	0.05	0.05

b vs a: $P < 0.001$, h vs i: $P = 0.007$, h vs g: $P < 0.05$, h vs f, e: $P < 005$, h vs d: $P = 0.01$
*The second set of values for conjugate $K1_{OAc+}$-TT was determined using OAc^+ K1 CP as the antigen.

Example 3

Induction of Poly α(2→9) NeuNAc and TT Antibodies (Table 3)

The homologous CP induced low levels of poly α(2→9) NeuNAc IgM antibodies. Neither the homologous nor the heterologous CP induced IgG antibodies.

All the conjugates elicited IgM antibodies after the first injection. These levels declined after the 2nd and 3rd injections of the MenC-TT and K92-TT conjugates and increased only after the first two injections of the K1-TT conjugates.

Only the MenC-TT elicited poly α(2→9) NeuNAc IgG antibodies after the first injection; all the conjugates elicited increases after the second and third injections. The highest

levels were elicited by MenC-TT>K92-TT>K1-TT. Similar to those observed with poly α(2→8) NeuNAc antibodies, the IgG antibody levels elicited by K92-TT_1 were higher than those elicited by K92-TT_2 but N.S.

TT antibodies were elicited by all the conjugates with booster responses after each injection similar to those reported for other conjugates using this protein as a carrier (data not shown) (Robbins, J. B., & Schneerson, R. (1990) J. Infect. Dis. 161, 821–832; Lagergard, T. et al. (1990) Infect. & Immun. 58, 687–694).

TABLE 3

Serum IgG and IgM antibodies (μg/ml) to the capsular-polysaccharide of group C *Neisseria meningitidis* (poly α (2→9) NeuNAc)

	Post-immunization geometric mean					
	IgM			IgG		
Antigen	1st	2nd	3rd	1st	2nd	3rd
K1	0.05	0.05	0.05[a]	0.05	0.05	0.05[e]
K1-TT_1	0.11	0.32	0.14[b]	0.05	0.14	1.24[f]
K1-TT_2	0.23	1.09	0.40[b]	0.05	0.97	3.32[f]
MenC	0.05	0.08	0.11[c]	0.07	0.10	0.05[e]
MenC-TT	2.26	0.89	0.53[d]	1.87	18.4	107.5[g]
K92	0.05	0.05	0.05[a]	0.05	0.05	0.05[e]
K92-TT_1	3.23	2.85	0.68[b]	0.05	0.70	21.4[h]
K92-TT_2	1.87	0.74	0.15[b]	0.06	1.71	15.9[h]

b vs a: P = 0.0001, d, vs c: P = 0.0004, c vs a: P < 0.001, f,g,h vs e: P = 0.0001, g vs f,h: P < 0.001

Example 4

Temperature-dependent Binding of IgG Antibodies (Table 4)

Binding to the two CP by IgG antibodies elicited by K92-TT, K1-TT and MenC-TT conjugates and *E. coli* K92 and *M. nonliguefaciens* cells was assayed at 22° C. and at 37° C. Reduction in binding at 37° C. of poly α(2→8) NeuNAc antibodies elicited by the K1-TT_2, *M. nonliquefaciens*, and K92-TT_1 was similar (≈40%). In contrast, there was only ≦10% reduction in binding of poly α(2→9) NeuNAc antibodies elicited by K1-TT_2, K92-TT_1, MenC-TT and *E. coli* K92 cells. These data are consistent with other results (Mandrell, R. E. & Zollinger, W. D. (1982) J. Immunol. 129, 2172–2178).

TABLE 4

Temperature-dependent binding of murine poly α(2→8) and poly α(2→9) NeuNAc IgG antibodies (percent binding at 37° C. compared to 22° C.)

	CP Used for ELISA	
Immunogen	poly α(2→8) NeuNAc	poly α(2→9) NeuNAc
K1-TT_2	41.8%	90.9%
M. nonliquefaciens cells	34.6%	N.D.*
K92-TT_1	49.1%	93.8%
E. coli K92 cells	N.D.	91.5%
MenC-TT	N.D.	100%

*N.D. Not detectable

Example 5

Poly α(2→8) NeuNAc Antibodies in Paired Maternal and Cord Sera (Table 5)

Most women at term had detectable IgM and IgG poly α(2→8) NeuNAc antibodies. The IgM and IgG levels of the Israeli women were higher than those of the women in Charlotte, N.C. (P=0.0001). As expected, the IgM poly α(2→8) NeuNAc antibodies in the cord were at trace or non-detectable levels. The geometric mean (GM) levels of IgG antibodies in the cord sera were significantly higher than those of the mothers from both regions. Most of the cord poly α(2→8) NeuNAc IgG antibodies were higher than those of the corresponding maternal sera (69/81).

TABLE 5

IgG and IgM antibodies to poly α(28) NeuNAc in paired human mother-newborn (umbilical cord) sera (Geometric mean)

		Maternal		Cord		Maternal IgG
Source	n	IgM	IgG	IgM	IgG	vs cord IgG
Charlotte, NC	36	0.35	26.9	0.03	32.9	P = 0.003
Sheba Medical Center, Israel	45	0.91	80.0	0.04	121	P = 0.0001

The levels of IgM antibodies are expressed as μg Ab/ml and the levels of IgG antibodies as percent of a high-titered adult serum (GH) as ELISA units.

Example 6

Passive Immunization

Either monoclonal or polyclonal antibodies, of human or animal origin, for passive immunization for prevention, or as adjunct therapy of systemic infections with organisms containing poly α(2→8) NeuNAc or poly α(2→9) NeuNAc surface antigens in an animal, including humans, may be produced by the above-described conjugate vaccines. (example: passive immunization of case contacts of group B meningococcal systemic infections including meningitis). Passive immunization, for both therapeutic and preventative purposes, has been carried out since the turn of the century. Passive immunization has been considered again for prevention of group B meningococcus systemic infections including meningitis, as well as other capsulated bacterial pathogens that cause systemic infections including the pneumococcus, Haemophilus influenzae type b, group B streptococcus and *E. coli* infections in hosts at higher risk than the general population including fetuses, newborns and patients with congenital or acquired immunodeficiencies (Patients with immunodeficiences may not be capable of producing protective levels of antibodies when injected with K92 and/or poly α(2→8) NeuNAc conjugate vaccines). The technique of passive immunization is taught by: Flexner (1913) J. Exp. Med. 17:553–570; Brahahm (1938) Proc. Soc. Exp. Biol. Med. 30:348; Raff et al. (1988) J. Infect. Dis. 157:118–126; Kim et al. (1985) Infect. Immun. 50:734–737; and Latson et al. (1988) Podiatr. Infect. Dis. 7:747–752.

Example 7

Further Uses of the Antibodies

Either monoclonal or polyclonal antibodies are prepared for diagnostic purposes or for the investigation of the developmental processes, pathogenesis, prevention, immunopathology, or immunologic responses of poly α(2→8) NeuNAc, poly (2→9) NeuNAc, or to poly α(2→8), α(2→9) NeuNAc alone, as a component or a complex molecule or of organisms containing these saccharides. The use of poly α(2→8) NeuNAc antibodies, especially of the IgG class, for use in developmental studies is illustrated in

the following articles: Husmann et al. (1990) J. Histochem. & Cytol. 38:209–215; Robbins & Schneerson (1990) J. Infect. Dis. 161:821–832). The above-described conjugate-induced antibodies may be derivatized or interacted with other substances to produce kits for diagnosis of diseases or identification of organisms containing poly α(2→8) NeuNAc or poly α(2→9) NeuNAc. Kits, containing polyclonal or monoclonal antibodies, are used worldwide for the diagnosis of systemic infections, including meningitis, or for asymptomatic carriers of *Neisseria meningitidis* as well as other capsulated bacterial pathogens. This use is reviewed in: Lim et al. (1990) J. Clin. Microbiol. 28:670–675; Cuevas et al. (1989) Ann. Trop. Med. Parasitol. 83:375–379; Ørskov et al. (1979) J. Exp. Med. 149:669–685.

Example 8

Active Immunization Against the Three Major Serogroups of *N. meningitidis*

Active immunization against the three major serogroups of *Neisseria meningitidis,* would include conjugate vaccines of group A along with the conjugate vaccines described-above. A trivalent polysaccharide-protein conjugate vaccine, capable of eliciting serum antibodies to groups A, B, and C meningococcal meningitis and thereby preventing most of the systemic infections, including meningitis, caused by *Neisseria meningitidis,* may be produced by this method using the above-described conjugates and a group A meningococcal conjugate. Group A meningococcal polysaccharide protein conjugates have been synthesized according to a published method (Chu et al. (1983) Infect. Immun. 40:245–256). Concurrent injection of more than one polysaccharide protein conjugate in animals and in humans has been shown to elicit protective levels of antibodies to each component at levels equal to those elicited by each conjugate injected separately (Schneerson et al. (1986) Infect. Immun. 52:501–518).

All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

What is claimed is:

1. An *Escherichia coli* K92 capsular polysaccharide and carrier protein conjugate, wherein said polysaccharide and said carrier protein are covalently coupled with a linker selected from the group consisting of adipic acid dihydrazide, diaminohexane, amino epsilon caproic acid, and N-hydroxysuccinimide acid anhydride-based heterobifunctional molecules, and further wherein said conjugate elicits the production of antibodies reactive with poly α(2→8)NeuNAc capsular polysaccharides of *Escherichia coli* K1 and group B *N. meningitidis* and with poly α(2→9) NeuNAc polysaccharide of Group C *N. meningitidis* microorganisms, and antibodies reactive with said carrier protein.

2. The conjugate according to claim **1**, wherein said carrier protein is immunogenic and is selected from the group consisting of albumin, chemically or genetically detoxified diphtheria toxin, tetanus toxoid, detoxified exotoxin A of *Pseudomonas aeruginosa,* detoxified *Staphylococcus aureus* toxin, synthetic polypeptides, bacterial outer membrane proteins, and viral proteins.

3. The conjugate according to claim **2**, wherein said carrier protein is tetanus toxoid.

4. The conjugate according to claim **1**, wherein said linker is adipic acid dihydrazide.

5. An *Escherichia coli* K92 capsular polysaccharide and tetanus toxoid conjugate, wherein said polysaccharide is covalently coupled to detoxified tetanus toxin with a linker selected from the group consisting of adipic acid dihydrazide, diaminohexane, amino epsilon caproic acid, and N-hydroxysuccinimide acid anhydride-based heterobifunctional molecules, and further wherein said conjugate elicits the production of antibodies reactive with poly α(2→8)NeuNAc capsular polysaccharides of *Escherichia coli* K1 and group B *N. meningitidis* and with poly α(2→9) NeuNAc polysaccharide of Group C *N. meningitides* microorganisms, and antibodies reactive with tetanus toxoid protein.

6. The conjugate according to claim **5**, wherein said linker is adipic acid digydrazide.

7. A poly α(2→8), α(2→9)NeuNAc containing polysaccharide and protein conjugate comprising a poly α(2→8), α(2→9)NeuNAc capsular polysaccharide component coupled to an immunogenic carrier protein component with a linker selected from the group consisting of adipic acid dihydrazide, diaminohexane, amino epsilon caproic acid, and N-hydroxysuccinimide acid anhydride-based heterobifunctional molecules, wherein said conjugate elicits the production of antibodies reactive with poly α(2→8) NeuNAc capsular polysaccharides of *Escherichia coli* K1 and group B *N. meningitidis* and with poly α(2→9)NeuNAc polysaccharide of Group C *N. meningitidis* microorganisms, and antibodies reactive with said carrier protein.

8. The conjugate according to claim **7**, wherein said carrier protein is immunogenic and is selected from the group consisting of albumins, chemically or genetically detoxified diphteria toxin, tetanus toxoid, detoxified exotoxin A of *Pseudomonas aeruginosa,* detoxified *Staphylococcus aureus* toxin, synthetic polypeptides, bacterial outer membrane proteins, and viral proteins.

9. The conjugate according to claim **8**, wherein said carrier protein is tetanus toxoid.

10. The conjugate according to claim **7**, wherein said linker is adipic acid dihydrazide.

11. A method of producing serum antibodies immunoreactive with poly α(2→8)NeuNAc-containing capsular polysaccharides of *Escherichia coli* K1 and Group B meningococci, antibodies reactive with poly α(2→9) NeuNAc-containing capsular polysaccharide of Group C meningococci, and antibodies reactive with a carrier protein, comprising the steps of:

a) providing a conjugate according to claim **1**, claim **5**, or claim **7**;

b) administering said conjugate to said mammal; and

c) boosting said mammal with said conjugate to produce said immunoreactive anti-polysaccharide antibodies and said anti-carrier protein antibodies.

12. The method according to claim **11**, wherein said carrier protein of the conjugate of step a) is immunogenic and is selected from the group consisting of albumins, chemically or genetically detoxified diphtheria toxin, tetanus toxoid, detoxified exotoxin A of *Pseudomonas aeruginosa,* detoxified *Staphylococcus aureus* toxin, synthetic polypeptides, bacterial outer membrane proteins, and viral proteins.

13. The method according to claim **12**, wherein said carrier protein is tetanus toxoid.

14. A method of immunizing a mammal to produce antibodies reactive with the capsular polysaccharide surface antigens of microorganisms containing poly α(2→8)